

In Re Application of:
Jay M. Short
Serial No.: 09/375,605
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PATENT
Attorney Docket No. DIVER1140-2

IN THE CLAIMS:

Please cancel claim 1-16 without prejudice.

Please add the following new claims:

- 21*
Insert
17. A method for obtaining a specified protein or bioactivity of interest encoded by DNA contained in a heterogeneous population of DNA from more than one source comprising:
- (a) screening more than one library, wherein the libraries contain a plurality of clones containing DNA from either a single source or a multiplicity of sources, for a specified protein or bioactivity;
 - (b) identifying a clone which is positive for the specified protein or bioactivity;
 - (c) introducing at least one mutation into the DNA contained in the clone of (b); and
 - (d) comparing the activity of a DNA expression product from (c) with the activity encoded by a non-mutagenized form of the DNA present in (b), wherein a difference in activity is indicative of an effect of introducing at least one mutation, thereby providing a specified protein or bioactivity of interest.
- 22*
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18. The method of claim 17, further comprising, prior to (c), obtaining DNA from a clone which is positive for the specified protein or bioactivity.
- 23*
19. The method of claim 18, wherein obtaining the DNA contained in the clone positive for the specified protein or bioactivity comprises contacting the clone with a complementary nucleic acid, or fragment thereof, thereby allowing hybridization of the clone DNA with the complementary nucleic acid and isolation thereof.
- 24*
20. The method of claim 19, wherein the complementary nucleic acid or fragment thereof comprises a solid phase bound hybridization probe.

21. The method of claim 17, wherein the mutation is introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis and any combination thereof.
22. The method of claim 17, wherein the mutation is introduced by error-prone PCR.
23. The method of claim 17, wherein the mutation is introduced by shuffling.
24. The method of claim 17, wherein the mutation is introduced by oligonucleotide-directed mutagenesis.
25. The method of claim 17, wherein the mutation is introduced by assembly PCR.
26. The method of claim 17, wherein the mutation is introduced by sexual PCR mutagenesis.
27. The method of claim 17, wherein the mutation is introduced by in vivo mutagenesis.
28. The method of claim 17, wherein the mutation is introduced by cassette mutagenesis.
29. The method of claim 17, wherein the mutation is introduced by recursive ensemble mutagenesis.
30. The method of claim 17, wherein the mutation is introduced by exponential ensemble mutagenesis.
31. The method of claim 17, wherein the mutation is introduced by site-specific mutagenesis.

32. The method of claim 17, comprising screening the heterogeneous population of DNA for a further specified protein or bioactivity, prior to exposing the DNA to mutagenesis.
33. The method of claim 32, wherein the screening is by hybridization with a nucleic acid probe.
34. A method for obtaining a protein having a specified bioactivity comprising:
screening a heterogenous population of DNA obtained from individual expression clones or a mixture of expression clones containing a plurality of activities which have been modified by mutagenesis, for a protein having one or more desired characteristics which can be the same or different from the specified bioactivity.
35. The method of claim 34, further comprising, prior to said mutagenesis, obtaining from the heterogeneous population, DNA which comprises DNA sequences coding for at least one common bioactivity, which can be the same or different from the specified bioactivity.
36. The method of claim 35, wherein obtaining the DNA comprises contacting the population with a complementary nucleic acid, or fragment thereof, thereby allowing hybridization of the DNA with the complementary nucleic acid and isolation thereof.
37. The method of claim 36, wherein the complementary nucleic acid, or fragment thereof comprises a solid phase bound hybridization probe.
38. The method of claim 33, wherein the protein is an enzyme.
39. The method of claim 38, comprising obtaining DNA from the heterogeneous population which exhibit a particular class of enzyme activity.